Paper No. 10

MICROBIAL DEGRADATION OF NICOTINE II - EFFECT OF ENVIRONMENTAL CONDITIONS AND COMPARISON OF VARIOUS AVAILABLE NICOTINE DEGRADING BACTERIAL ISOLATES

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ABSTRACT

Under improved conditions, nicotine-degrading bacterial isolates which did not form blue pigment tolerated and metabolized more nicotine. These conditions consisted of the use of a high-speed rotary shaker and Erlenmyer flasks modified by forming two elongated indentations in opposite walls of the flask to function as baffles. Nicotine-degrading bacterial isolates obtained from various investigators were compared with our own cultures using conventional biochemical tests. Freeze-dried cultures grown in two different media also were compared by an infra-red spectrophotometric technique. Oxidation of various compounds by the previously reported six nicotine-degrading isolates was investigated using a manometric technique. Bacterial isolates which did not form blue pigment in nicotine-containing medium lost their ability to oxidize nicotine after being lyophilized. The same isolates, freshly harvested, oxidized nicotine at a faster rate than those which formed blue pigment. When the isolates were grown in a medium containing conventional sources of carbon and nitrogen instead of nicotine, only one oxidized nicotine to a small extent. However, all isolates oxidized nicotine readily when grown in a conventional medium supplemented with nicotine.

REVIEW BY A. B. CLARKE

This was a continuation of work reported last year on the microbial degradation of nicotine. Certain improvements were made to oxidize nicotine more readily. A high speed shaker and a modified Erlenmyer flask increased aeration to step up the degradation of nicotine. Cultures obtained from various investigators were compared using the improved technique. (Table 1)

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TABLE 1

CULTURAL CHARACTERISTICS OF BLUE PIGMENT-FORMING, NICOTINE-DEGRADING BACTERIAL ISOLATES

Isolate	#1	#4	P-34	P.S.	KD	LiC	Cer
Source	Soil	Tobacco Seed	Rittenberg	Sguros	Decker	Stone	Stone
Morphology (Nicotine Medium)	----------Short Rods, Some Coccoid Forms----------						
Size (μ)	-Coccoid Forms 0.4 x 0.5, Short Rods 0.5 x 0.7 to 0.6 x 0.8----------						
Color:							
Nutrient Agar	Off-White	Off-White	Yellow	Pale Yellow	Pale Yellow	Off-White	Off-White
Nicotine Medium	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Gram Reaction	-	-	-	-	-	-	-
Nitrate Reduction	+	+	-	+(weak)	+	+	-
Litmus Milk (pH/Digestion)	Acid/-	Alkaline/+	Alkaline/-	Alkaline/-	Alkaline/+	Alkaline/+	Alkaline/
Casein Hydrolysis	+	+	+	-	+	+	-
Gelatin Liquefaction	Rapid	Rapid	-	Slow	Rapid	Slow	Slow
Starch Hydrolysis	-	-	-	-	+	-	-
H_2S-Formation	+	+	-	-	-	+	-
Indol Formation	-	-	-	-	-	-	-
Acetylmethylcarbinol	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Nicotinic Acid	-	-	-	-	+	-	-
Glucose, Sucrose Fructose (growth/pH)	+/Acid	+/Alkaline	+/Alkaline	+/Alkaline	+/Alkaline	+/Alkaline	+/Alkaline

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Oxidation of nicotine-related compounds grown in Sguros medium are shown in Table 2. Those bacterial isolates which lyophilized did not form blue pigment and did not oxidize nicotine. However, if the isolates are fresh, those forming blue pigment are slower in oxidizing nicotine than are those forming another color.

TABLE 2

OXIDATION OF NICOTINE-RELATED COMPOUNDS
BY WASHED SUSPENSIONS OF ISOLATES NOS. 1-6
GROWN IN SGUROS' MEDIUM

$-\Delta O_2$ (µl/mg. dry cell wt./2 hrs.)

	Isolate Numbers					
Substrate (0.02 M)	1	2	3	4	5	6
Pseudo-Oxynicotine	15.7	198.5	264.5	20.2	16.2	290.0
Oxynicotine	24.1	42.5	26.1	11.7	30.2	62.9
Myosmine	22.0	17.4	31.0	26.3	38.0	15.7
Nicotyrine	-	-	-	-	-	-
Nicotine-Ethylene Oxide Complex	-	-	-	-	-	-

The oxidation of nicotine grown in different media is shown in Table 3. Notice that nicotine is oxidized in Sguros' Medium. Nutrient Broth is not effective unless supplemented with nicotine.

TABLE 3

THE OXIDATION OF NICOTINE (0.02 M)
BY WASHED SUSPENSIONS OF BACTERIAL ISOLATES NOS. 1-6
GROWN IN DIFFERENT MEDIA

$-\Delta O_2$ (µl/mg. dry cell wt./2 hrs.)

	Isolate Numbers					
	1	2	3	4	5	6
Sguros' Medium	53.1	231.5	294.5	65.5	263.5	193.5
Nutrient Broth	-	-	27.8	-	-	-
Nutrient Broth + Nicotine	28.9	48.2	203.5	50.4	165.0	163.5
Nutrient Broth followed by 5 hours in Nicotine	-	-	23.0	-	-	-

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